

31 AccuTOF-DART	Page 1 of 3
Department of Forensic Science	Amendment Designator:
CONTROLLED SUBSTANCES PROCEDURES MANUAL	Effective Date: 17-December-2007

31 AccuTOF-DART Mass Spectrometry

31.1 Introduction:

- 31.1.1 Direct Analysis in Real Time (DART) is an atmospheric pressure ionization technique that can analyze solids, liquids and gases by placing the test material into a heated gas flowing through the sampling area. Ionization occurs from the surface of the sampling medium. Coupling of this ion source with an accurate mass time-of-flight mass spectrometer (AccuTOF) gives quick and simple analyses with little to no sample preparation.
- 31.1.2 While ionization can be done in both positive and negative mode, the large majority of drugs give usable spectra in positive ion mode. Ionization in positive ion mode is accomplished by charging a heated helium gas stream, forming metastable helium ions which react with ambient water vapor, producing hydronium ions which subsequently react with the sample molecules to induce ionization. The mechanism of positive and negative ion production with the DART is discussed by Cody, *et. al.* (See Reference 31.3.1)
- 31.1.3 In general, DART ionization produces spectra with a characteristic peak at the protonated or deprotonated molecule. These ions are measured at their exact mass in the AccuTOF mass spectrometer. Elemental composition calculations, based on empirical formulas, can be performed on these ions to determine whether they fall within a specified range, usually measured in millimass units (mmu), of a known compound. While accurate-mass spectra have an inherent specificity, full identification is difficult if the possibility of an isomer exists. To increase the specificity of the technique, the voltages on the orifice1 of the AccuTOF are varied, which can produce spectra with extensive fragmentation by in-source collision-induced dissociation (CID). This can be accomplished by utilizing the function switching mode of the AccuTOF operating software. Function switching allows for simultaneous collection of spectra at several different orifice1 voltages (See Reference 31.3.2). Higher orifice1 voltages generally result in more characteristic ions being produced. The combination of accurate mass measurement of the protonated molecule and characteristic CID fragmentation allows for the production of spectra that can be used as part of an identification scheme for drugs of abuse.

31.2 General Drug Screening Method:

31.2.1 Instrumentation, Instrument Parameters and Materials

31.2.1.1 The DART ion source is coupled to a JEOL AccuTOF™ mass spectrometer (JMS-100LC) and operated in positive-ion mode. This system is controlled by “Mass Center” software.

31.2.1.2 Method parameters are listed in Table 6 below.

Table 6: Method Parameters for DART_POS_SWITCH_02 (General Screening Method – Positive Mode)

Parameter	Setting	Parameter	Setting
Ion Guide Peak Voltage	600V	Mass Range	66 – 600 Daltons (Da)
Orifice1 Voltage	Variable (20V, 30V, 60V, 90V)	Helium Flow Rate	4.0 L/min.
Spectrum Recording Interval	Every 0.25 seconds	Gas Heater Temperature	275 °C
Orifice2 Voltage	Approximately 5V	Discharge Electrode Needle	4000V
Ring Lens Voltage	Variable Approximately 3-6V	Electrode1	150V
Orifice1 Temperature	80 °C	Electrode2	250V

31.2.1.3 Internal mass calibration is accomplished using a dilute solution of polyethylene glycol (PEG) 600 (Chem. Service, West Chester, PA) in methanol.

31 AccuTOF-DART	Page 2 of 3
Department of Forensic Science CONTROLLED SUBSTANCES PROCEDURES MANUAL	Amendment Designator:
	Effective Date: 17-December-2007
<p>31.2.1.4 Separate tune files are established where the only difference is in the orifice1 voltage.</p> <p>31.2.1.5 Cleaned glass melting point tubes</p> <p>31.2.1.5.1 It is necessary to clean the melting point tubes prior to use as a sampling device. The procedure below removes the majority of the dioctyladipate contaminant from the tubes. It does not, however, remove ALL of this contaminant from every tube. A more exhaustive cleaning method may need to be employed if this peak interferes with analyte peaks of interest.</p> <p>31.2.1.5.2 Capillary Tube Cleaning Procedure:</p> <ul style="list-style-type: none"> • Remove tubes from plastic container and place into beaker, closed end down. • Squirt acetone onto tubes while moving them around in the beaker to attempt to rinse as well as possible. • Remove tubes and discard acetone. • Shake tubes slightly to remove excess acetone. • Place tubes back in beaker, closed end down. • Squirt, vigorously, with methanol, moving tubes to attempt to squirt down all tubes. • Remove tubes, discard methanol. • Repeat methanol wash. • Place tubes, closed end UP, in another beaker and place in the vacuum oven. • Turn on vacuum oven and dry tubes until methanol is evaporated. • Transfer tubes to another dry beaker, closed end down. • Remove several tubes at a time and hold them in the HOT effluent of a heat gun for several seconds. • Repeat previous step until all tubes have been "heat treated". • Place tubes into a clean, screw-top vial until ready for use. <p>31.2.2 Procedure:</p> <p>31.2.2.1 Although samples may be run in any chemical state, it is recommended that powders, tablets and capsules be dissolved in a suitable solvent (e.g., methanol, methylene chloride, ammonia saturated chloroform).</p> <p>31.2.2.2 In general, samples are run by dipping the closed end of the glass melting point tubes into the sample solution and then immediately inserting the tube into the DART gas stream for several seconds. Replicate samplings (2-3) are recommended within the data file to more fully represent spectra of the analytes of interest. Let the melting point tube cool briefly between samplings in order to achieve better consistency when sampling volatile solvents. Other sampling methods (e.g., solids, plant materials, gases, dried liquids) may be run after consultation with primary instrument operator.</p> <p>31.2.2.3 Bring DART and AccuTOF out of standby mode, load DART_POS_O1_20V tune file and set the AccuTOF to "Operate". For more detailed instructions, see Reference 31.3.3.</p> <p>31.2.2.4 PEG 600 calibrant solution shall be run within each data file. Replicate samplings (2-3) are recommended for proper internal mass calibration.</p> <p>31.2.2.5 After you are finished collecting data, turn the DART to standby to conserve helium.</p> <p>31.2.2.6 Averaged, background subtracted, centroided spectra are used for all data reduction.</p> <p>31.2.2.7 Mass calibration is performed utilizing the PEG600 spectra in the 30V function.</p>	

31 AccuTOF-DART	Page 3 of 3
Department of Forensic Science CONTROLLED SUBSTANCES PROCEDURES MANUAL	Amendment Designator:
	Effective Date: 17-December-2007
<div data-bbox="428 294 1485 352"> <p>31.2.2.7.1 Maximum error should be less than 0.003 mmu and standard error should be less than 0.001.</p> </div> <div data-bbox="428 386 1528 478"> <p>31.2.2.7.2 The polynomial fit must be set to 3 or 4. A maximum of two data points may be removed to improve the calibration curve. Care should be taken not to remove data points at either end of the calibration table so as not to decrease the effective mass range of the data file.</p> </div> <div data-bbox="428 510 1437 569"> <p>31.2.2.7.3 Data files, which include calibration data, are archived. Overwriting the working calibration file is acceptable since they can be regenerated.</p> </div> <div data-bbox="428 600 1458 630"> <p>31.2.2.7.4 Internal mass calibration should be applied to all functions where data will be used.</p> </div> <div data-bbox="313 661 599 690"> <p>31.2.2.8 Data processing</p> </div> <div data-bbox="428 722 1463 810"> <p>Spectra should be saved in JEOL-DX format for use by other data reduction software (e.g., SearchFromList, Elemental Composition Workshop). Repeat as necessary for spectra from other orifice1 functions.</p> </div> <div data-bbox="313 842 1531 900"> <p>31.2.2.9 Return AccuTOF and DART to standby mode. Do not turn the DART gas off before the temperature is down.</p> </div> <div data-bbox="217 932 522 961"> <p>31.2.3 Data Interpretation:</p> </div> <div data-bbox="313 993 1490 1052"> <p>31.2.3.1 Data from the 20V function is used to obtain information regarding molecular weight and should be searched against the Drug Neutral Mass library.</p> </div> <div data-bbox="313 1085 1547 1207"> <p>31.2.3.2 Data from functions with higher Orifice1 values result in greater fragmentation, and should be searched against the single component standard and/or the drug preparation libraries for those voltages. Poly-drug spectra can lead to complex spectral interpretation. Care must be taken to evaluate search results using both types of information.</p> </div> <div data-bbox="313 1239 1398 1268"> <p>31.2.3.3 Comparison of spectra to in-house library search results should be included in the case file.</p> </div> <div data-bbox="313 1299 1502 1358"> <p>31.2.3.4 To report the identity of a drug indicated by this screening method, confirmation utilizing the normal analytical scheme is required.</p> </div> <div data-bbox="151 1390 350 1419"> <p>31.3 References:</p> </div> <div data-bbox="217 1451 1518 1509"> <p>31.3.1 Cody RB, Laramée JA, Nilles JM, Durst HD. "Direct Analysis in Real Time (DART) mass spectrometry" <i>JEOL News</i> 2005; 40(1): 8-12.</p> </div> <div data-bbox="217 1541 1523 1602"> <p>31.3.2 JEOL, Inc. Application note: <i>LC/MS: Identification of unknowns by combining exact mass measurement with the NIST 02 Mass Spectral Database Similarity Search</i>, www.JEOL.com/ms/accutof.html, January 2003.</p> </div> <div data-bbox="217 1633 810 1663"> <p>31.3.3 DFS AccuTOF-DART Operating Instructions.</p> </div> <div data-bbox="1484 1667 1547 1692"> <p>◆End</p> </div>	